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FAT UPON THE METABOLISM OF CALCIUM
AND PHOSPHOROUS.

THESIS FOR THE DEGREE OF DOCTOR OF PUBLIC HEALTH

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May 30 th, 1923.

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A REVIEW OF THE LITERATURE.

Until a few years ago the metabolism of the elements calcium and phosphorous had been studied quite extensively, but without any startling results. The studies on calcium were undertaken mainly from the clinical interest in such diseases as tetany and rickets, and conditions such as malnutrition, fasting and parathyroidectomy. Studies on phosphorous were stimulated by a broader interest due to the fact that this element so frequently appears in the diet in combination with fats such as lecithin, and with proteins such as casein,vitellin and nucleic acid. This interest was still further broadened by the biological fact that casein the chief protein of milk,the food of infant mammals, and vitellin the chief protein of eggs, are both phosphorized proteins. It was from this latter fact that the interesting discussion arose as to the relative merits of organic versus inorganic compounds of phosphorous for the body's metabolism. In both cases the phosphorous itself, sofar as is known, is present mostly in the completely oxidized form of phosphoric acid, differing only in its chemical relationships. The question to be answered was whether organic and inorganic phosphorous compounds could serve equallly well all the conditions of life. In other words were they equally useful for all the purposes for which the body needs phosphorous.

W.S.Hall (1) in 1896 appears to have been the first to have investigated this question. He concluded from his work that the organically bound phosphorous was more easily assimilated than the inorganically bound phosphorous.Up to the year 1909 all the investigators of the question had come to practically the same conclusion. In that year Hart, McCollum and Fuller (2) published the results of their work showing that hogs died on low phosphorous rations but survived if calcium phosphate was added. In the same year McCollum published (3) his "Nuclein Synthesis in the Animal Body",showing that the inorgainc phosphates suffice for all nutritive needs of the body.

This paper also reported the first successful growth experiments with a food supply which was considered at that time as essentially a mixture of purified food stuffs, every one of which could be named. McCollum had been working on the problem of the successful feeding of simplified diets of carbohydrates, fats, proteins, and salts. He apparently had obtained success by the introduction of a variety of food stuffs into the diet. Thus the protein used was edestin from hemp seed and zein from maize; starch was obtained from corn and wheat; sugar from lactose, glucose and saccharose; fat from butter and bacon and also cholesterol; and the inorganic phosphate was tri-calcium phosphate. On this diet his animals lived, but when his work was repeated by Osborne and Mendel (4) their results were contradictory. McCollum began to reinvestigate the problem to find what had caused the diversity of results. As will be shown below it was from this work of his that the existence of "Fat-Soluble A" became established.

In the mean time Stepp in 1909 published his work. Mice fed on diets extracted with either and chloroform died within thirty days, whereas controls fed on the same without the either and chloroform extraction were living after a period of six weeks. He also worked with pigeons and finally concluded that only certain lipins were necessary to supplement successfully a diet of purified food substances of the hitherto recognized group of foods. (5).

(6)Later Osborne and Mendel reported their preparation of protein from milk and of obtaining successful growths with its use. Hopkins found that he (7) could obtain growths with diets hitherto unsuccessful by the simple addition of a few cc of milk. For five years the interpretations of these results remained vague and obscure. In 1914 McCollum and his co-workers demonstrated that growth in all of these experiments was due to the presence, in the diet in addition to the carbohydrate, fat, protein and salts, of two organic food substances, which being still unknown, were called "Fat-soluble A" and "Water-soluble B". (8).

The former had been present in the butter fat and the latter had been present as

an impurity in the lactose of the diet used by McCollum in the above described experiment. Osborne and Mendel had used another kind of fat which was lacking in the the growth promoting vitamin and their sugar did not contain Water soluble B. This in brief is the history of the discovery of Fat soluble A.

The history of the vitamin associated with the disease of rickets dates back to the work of Mellanby (9), published in 1919. He used two hundred pups in his investigation of the importance of diet in the production of rickets. He based his diagnosis upon the x-ray, the calcium estimation of the bones and the histological examination.

He worked with the following four diets, any one of which would produce rickets:-

(1) Whole milk 175 cc., oatmeal, rice, and 1/2 gram NaCl.

(2) Whole milk 175 cc., bread ad lib.

(3) Separated milk 250-350 cc., bread 70 % wheaten ad lib., linseed oil 5-15 cc, yeast 5-10 grams, orange juice 5 cc., NaCl 1/2 gram.

(4) Separated milk 175 cc., bread 70 grams ad lib., linseed oil 10 cc., yeast 10 grams, NaCl 1 to 2 grams.

With diet number one he found that the addition of 500 cc of whole milk would prevent the development of rickets. With number two he found that the addition of meats or their watery or alcoholic extracts prevents rickets. The use of malt, orange juice, and yeat were not important in the protection of the dogs against rickets. However with fats he found that their protective effects were graded with cod liver oil as the best and linseed oil the poorest.

He concluded that rickets was a deficiency disease developing in consequence of the absence of some accessory food factor or factors. Of the then known accessory food factors, the results with yeast and orange juice showed that the lack of water soluble B and antiscorbutic substances could be eliminated as a cause. On the other hand the antirachitic factor was found to be

to be distributed similarly to fat soluble A. However he noted several points against the same distribution. Thus rickets develops best in rapidly growing animals and as McCollum had shown Fat soluble A is a growth promoting substance, so that it would be paradoxical to say that it was absent. Also he noted that the presence of varying amounts of antirachitic substance in vegetable oils was against the same distribution. Nevertheless he was still inclined to identify the fat soluble A, or some substance having a similar distribution, as the specific anti-rachitic substance. His most striking point was the demonstration of the protective function of butter fat and cod liver oil against a rickets like disease found in growing pups.

According to Trousseau(10) the use of cod liver oil in rickets dates back to Bretonneau(11) who used it first in 1829. The first to theorize upon its action was Jacobi,(12) who in 1898 suggested that the effect of cod liver oil might be explained by attributing to it a per centage, tho ever so small, of some organic tissue juice of a nature and efficacy comparable with that of the thyroid ~~gal~~ gland. The experiences of Schabad(13) in 1909 and 1910 led him to conclude that altho phosphorous exerted no influence upon calcium retention in normal children, yet when combined with cod liver oil, it favoured calcium retention in the rachitic child.

For a while the experimental data of Mellanby was accepted without clinical challenge. In 1920 Hess and Unger(14) published their results in which they showed that children fed on skim milk powder together with cane sugar, orange juice, yeast and cotton seed oil grew fairly well and without the development of rickets. Their conclusions were erroneous due to the fact that their skim milk powder was not free from Fat soluble A.(15). In spite of their previous experimental data on the therapeutic value of cod liver oil (16) they concluded from subsequent studies on light rays that rickets was due mostly to such unhygienic factors as the absence of sunlight.

The whole subject was by now in a rather confused state. No one had been able to separate the anti rachitic substance from Fat soluble A, and still Fat soluble A was not able to prevent or cure rickets. At this point McCollum and his co-workers decided to investigate the problem from a new point of view and by another method. They kept all their experimental animals in one room so that hygienic factors would play an unimportant role, and they systematically varied the the diet with respect to one or more factors. The diagnosis of rickets was always confirmed by a histological examination at autopsy.

From their experience with extensive studies of nutrition problems with a large rat colony, they were already in possession of the knowledge that certain animals whose diets were faulty in one or more respects, developed a condition which presented the gross appearance of rickets. Early they showed that when the diet was satisfactory in every respect except for a deficiency of calcium, the content of phosphorous and of Fat soluble A being near the optimal, changes take place in the skeleton which bear a fundamental resemblance to rickets. The same diet with the addition of calcium carbonate produced no lesions. The presence of as little as one per cent of cod liver oil in this calcium deficient diet would prevent the formation of lesions. Butter fat in as large a quanity as twenty per cent would not protect the animal against the condition produced by this diet. Diets satisfactory in every respect save for a deficiency of Fat soluble A produce pathological lesions, but neither a lack of Fat soluble A nor of cod liver oil can by themselves cause rickets. With diets deficient in phosphorous and free from the anti rachitic substance, young rats will have skeletons which bear fundamental resemblaces to rickets. They thus concluded that three factors operate in the etiology of rickets- the content of calcium, of phosphorous, and of vitamins. in the diet.

Studying these three factors more closely McCollum and his co workers concluded that the ratio between the concentration of calcium and phosphorous in the diet may, within certain limits, be of greater importance than the absolute amounts of these substances which the diet contains, and in the light of

this important discovery they were able to put a very different interpretation upon the findings of Sherman and Pappenheimer. These authors had thought that the production of rickets in their experiment had been due to the deficiency of phosphorous. Due to the above work of McCollum it is now thought to have been due to an unfavorable ratio between the calcium and phosphorous in the absence of a sufficient amount of the organic factor which plays a role in the production of the syndrome.

Working along these lines it was shown by Howland and Kramer that the blood serum of normal children and of rachitic children possesses the same amount of calcium, whereas the amount of phosphorous showed marked variations in health and disease. They found that the administration of cod liver oil to children with a lowered content of phosphorous in their serum caused the amount amount of phosphorous to increase to its normal figure. It was, therefore, suggestive that the increased concentration of phosphorous in the serum may be the cause of the resumption of the deposition of calcium phosphates in the long bones of animals suffering from rickets.

From the above work it was no longer doubted that cod liver oil was efficacious in rickets. However the nature of the active principle in the oil remained unknown. It had been stated that the fat soluble A content of cod liver oil was responsible for its beneficial effect. McCollum had already published certain evidence which led him to believe that the protection against rickets which cod liver oil affords is not due to Fat soluble A.

In the mean time Hopkins had shown that Fat soluble A is readily destroyed by oxidation. Following this lead McCollum and his co-workers oxydized cod liver oil for from twelve to twenty hours at 100 C. by blowing air though it. Cod liver oil so treated loses its curative power for xeroopthalmia. On the other hand it was just as effective in curing rickets produced in rats as the untreated oil. Thus they demonstrated quite completely that the anti rachitic effect of cod liver oil was not due to its content of fat soluble A, but that it was due to some other factor which the oil

contained. From its mode of action and the very small amounts of it which were required to exert its directive influence on metabolism, they concluded that it must be classed with the group of essential nutritive principles.

The above briefly summarizes the growth our knowledge about the effect of of cod liver oil and butter fat on the metabolism of calcium and phosphorous. It was mostly obtained from growth experiments on short lived animals. Histological examinations were carried out only for verification of diagnosis. However these examinations have opened up new lines of thought and future progress seems to be closely linked with this line of procedure.

THE HYPOTHESIS.

Growth in the length of the long bones takes place at the junction of the shaft and the head. The head is composed of cartilage cells which arrange themselves in columns parallel to the long axis of the bone and is called the proliferative cartilage. It is here that the lime salts are deposited forming "The provisional zone of calcification". This junction of the shaft with the cartilage forms a straight line. In the rachitic bone it is irregular due to the fact that this provisional zone of calcification is lacking. This condition allows cartilage to persist where it should be destroyed, and the cartilage, itself, is invaded by large tufts of blood vessels from the shaft which irregualarly destroy it. Osteoid tissue is not calcified, accumulates at points of stress and strain.

In the rachitic animal the deposition of lime salts and the provisional zone of calcification reappear upon the addition of cod liver oil to the diet, and the only change found in the blood serum is an increased amount of phosphorous, the calcium remaining the same.

Since this deposition of calcium takes place with out any increase of the calcium in the diet, the origin of it remains ~~t-e-~~ a source of conjecture. Doctor McCollum has noted for a long time, that in growing rats, the shaft of the long bones presents an osteoporotic condition after treatment with cod liver oil. It was assumed that the oil exerted an osteoclastic action upon the shaft of the long bones setting free the calcium salts which are needed at the epiphyses. This would explain the porosity of the shaft of the long bones. If this was so it was thought that the amount of calcium excreted would be increased upon the addition of cod liver oil to the diet.

The following experiment was arranged to test the hypothesis.

THE EXPERIMENT.

The experiment was divided into four periods of two weeks each.

Period One.

Two weeks on basal diet. (Three diets, changing each day by day.)

Calcium and phosphorous determinations for the period.

Period Two.

Two weeks on basal diet supplemented with butter fat.

Calcium and phosphorous excretions for the period.

Period Three.

Two weeks on basal diet alone.

Calcium and phosphorous excretions for the period.

Period Four.

Two weeks on the basal diet supplemented with cod liver oil.

Clacium and phosphorous excretions for the period.

Period Five.

Two weeks on basal diet to see if body would store calcium.

THE INTAKE OF CALCIUM AND PHOSPHORUS.

BREAKFASTS

Orange for vitamin
Bacon or ham
Cream of wheat with raisins, eaten with syrup
Crackers
Coffee. Black if possible, or a little [illegible] in it.

LUNCHES.

(1)

- Chicken soup
- Crackers
- Canned baked beans
- Pickles
- Apple sauce

(2)

- Soup
- Canned peas
- Crackers
- Pine apple sauce

(3)

- Corn cooked with bacon
- Peas
- Crackers
- Baked apple with raisins, no cream.

DINNERS.

(1)

- Broiled lean steak
- Baked potato
- Pickles
- Crackers
- Apple pie or baked apple.

(2)

- Cold tongue or ham
- Froed potatoes in bacon fat
- Crackers
- Pickles
- Pine apple sauce

(3)

- Beef balls without fat
- Bacon fat cooked in water
- Baked potatoes
- Corn
- Pear sauce

If apples, pineapples and pears become tiresome by themselves, they could be put together and served as a salad. Let the juice of the pineapple serve as the dressing.

Cold boiled potatoes and cold peas can be combined and seasoned with French dressing and served as salad.

Weigh and tabulate the amount of everything eaten per day.

• Diets planned by Miss Simmons.

FOODS TO BE USED IN CARRYING OUT THE DIETS.

Canned corn

Canned peas

Canned baked beans

Clear beef steak

Tongue

Crackers

Marmalade

Canned chicken soup

Apples

Small orange

Potato - plained boiled, baked, or fried in bacon fat.

Canned pears or pine apple and apple sauce for dessert.

Apple pie

Pickles and olives

Cream of wheat with raisins or dates

Coffee but no cream

Oatmeal or cereals cooked in water

Bacon or ham

Baked apples

Catsup - small amount.

Can have sugar- hard candies, but no choclate, butter creams, or vanilla creams.
No Hersey Choclate.

Breakfast thruout the entire period consisted of about six [illegible] sliced pieces of bacon, one orange, and four or five saltines. Lunches thruout the entire period consisted of one can of chicken soup and about one dozen saltines. This leaves only the evening meal requiring a detailed description.

Period of Basal Diet.

first day
1 ladel of hominy grits
4 slices bacon
1 slice tongue
[illegible] crax

second
2 potatoes
2 slices tongue
6 saltines

third
6 slices bacon
2 slices tongue
1/4 can corn
1 apple

fourth
steak $\frac{1}{4}$#
5 potatoes
1 ladel hominy

fifth
1 pc. ham $\frac{1}{4}$#
1 ladel corn
3 potatoes

sixth
6 slices bacon
5 potatoes
1 ladel peas
1 can chicken soup

seventh
3 slices ham $\frac{1}{4}$#
1 ladel peas
4 potatoes
1 orange

eight
ham $\frac{1}{4}$#
4 crax
6 stuffed olives

nninth
6 slices bacon
1 ladel corn
1 ladel peas
4 potatoes
2 slices apple pie

tenth
Ham $\frac{1}{4}$#
1 aldel peas
3 potatoes
1 ladel of corn
1 baked apple

eleventh
3 beef croquets
3 baked potatoes
2 boiled "

twelfth
ham $\frac{1}{4}$#
1 aldel rice
4 potatoes

thirteenth
steak $\frac{1}{2}$#
3 potatoes
1 ladel peas
1 baked apple

fourteenth
1 can soup
2 ladels of corn
3 slices bacon

Period of basal diet plus butter fat.

first
4 slices tongue
1 ladel corn
2 slices bacon
6 crax

second
2 slices bacon
1 ladel corn
1 pc apple pie

third
2 baked potatoes
ham $\frac{1}{4}$#
2 baked apples

fourth
1 pc steak $\frac{1}{2}$#
2 ladels of corn
6 crax

fifth
1 pc steak $\frac{1}{2}$#
3 potatoes
1 ladel of peas

sixth
roast beef $\frac{1}{4}$#
ham 1/8 #
3 potatoes

seventh
ham $\frac{1}{4}$#
3 potatoes
1 ladel peas

eighth
ham $\frac{1}{4}$#
3 potatoes
1 ladel peas

ninth
ham $\frac{1}{4}$#
1 ladel peas
6 saltines

tenth
Steak $\frac{1}{4}$#
3 potatoes
6 crax
1 apple

eleventh
ham $\frac{1}{4}$#
3 potatoes
1 ladel corn
" peas
1 apple

twelfth
ham $\frac{1}{4}$ #
3 potatoes
1 ladel peas

butter eaten 1 #

ham $\frac{1}{4}$ #
1 ladel rice
1 ladel peas
2 potatoes

ham $\frac{1}{4}$ #
3 potatoes
1 ladel corn

Basal diet alone.

First
fried ham ½ #
6 poatatoes
1 ladel corn
2 pieces apple pie

second
steak ½
6 slices bacon
2 ladels corn
6 potatoes
6 prunes
1 apple

third
1 can soup
ham ½ #
1 ladel [illegible]
6 potatoes
2 pcs apple pie
prunes

fourth
ham ½ #
1 ladel peas
2 ladels corn
8 potatoes
2 baked apples

fifth
ham ½ #
8 potatoes
1 ladel corn
pickles
2 pcs hard candy
1 pc apple pei

sixth
1 can soup
ham ½ #
2 slices pie
2 ser. cherries
1 pear

seventh
steak ½ $
fried potatoes
6 potatoes
1 ladel corn
1 ladel peas
1 apple
1 orange

eighth
ham ½ #
6 potatoes
6 prunes
6 olives
1 ladel peas
2 appples

ninth
beef ½ #
1 ladel corn
6 prunes
6 olives
6 pottatoes
2 pcs apple pie

tenth
~~ham~~ steak ½#
fried potatoes
6 potatoes
1 ladel corn
1 orange
2 pieces apple pie

eleventh
beef roast ½ #
1 ladel hominy
1 ladel peas
6 olives
6 prunes
2 baked apples

teelftn
steak ½ #
1 ladel corn
6 potatoes
6 prunes
1 ladel peas
2 baked apples

Thirteenth
steak ½#
ham ½ #
potatoe chips
6 potatoes
1 ladel peas
1 apple
1 slice pine apple
7 cherries
1 pear

Basal diet and cod liver oil.

first
beff $\frac{1}{2}$
6 potatoes
1 ladel peas
6 prunes
1 ladel corn
6 olives
2 apples

second
bacon 6 slices
2 ladels corn
6 potatoes
2 apples

third
steak
2 ladels corn
1 ladel peas
6 olives
6 prunes
4 potatoes
2 ~~pieces~~ apples

fourth
ham $\frac{1}{2}$
1 ladel corn
6 potatoes
1 orange
1 piece apple pie

fifth
beef croquets 6
potatoes 4
1 ladel peas
2 apples

sixth
steak $\frac{1}{2}$ #
potatoes 4
1 ladel corn
6 olives
6 prunes
2 apples

seventh
beef $\frac{1}{2}$ #
5 potatoes
1 ladel corn
1 ladel peas
1 orange

eighth
ham $\frac{1}{2}$ #
6 potatoes
1 ladel peas
2 pieces apple pie

ninth
steak $\frac{1}{2}$
5 potatoes
1 ladel corn
6 olives
6 prunes
2 apples

tenth
ham $\frac{1}{2}$
6 poatatoes
1 ladel peas
2 pcs apple pie

eleventh
beef $\frac{1}{2}$ #
potatoes 6
1 ladel corn
6 olives
6 prunes
2 oranges

twelfth
ham $\frac{1}{2}$ #
potatoes 5
1 ladel corn
1 ladel peas
2 pcs apple pie.

THE OUTPUT OF CALCIUM AND PHOSPHORUS.

[illegible]

CALCIUM.

URINARY.

Make the urine neutral or slightly acid and filter. Take 200 cc of the filtered urine. Add 10 cc of 2.5% oxalic acid. Stir and run in slowly 5 cc of 20% sodium acetate. Shake vigorously 10 minutes. Filter and wash free from chlorides with .5% ammonium oxalate solution. The filter paper and precipitate are then burned & dried to constant weight.

FECAL. Ignite the weighed amount of dried feces in a crucible to a white ash. Dissolve with hydrochloric acid. Make the volume up to 75-150 cc. Just acidify to litmus and add 10 drops of concentrated HCl. Add 10 cc of 2.5% oxalic acid. Close with a rubber stopper and shake ten minutes. Add excess of 3% ammonium oxalate. Add 10 cc of 20% sodium acetate solution. Stopper and shake 10 minutes. Filter and burn to constant weight.

PHOSPHORUS.

[illegible] AND FECES.

Place 10 cc of urine or an amount of feces equal to 50 mgs of P_2O_5 into a Kjeldahl flask. Add 10 cc of a mixture of equal parts of nitric and sulphuric acid. [illegible] tion [illegible] continue the digestion for 10 - 15 minutes. [illegible] acidify with nitric. Add 15 grams of ammonium nitrate. Heat on water bath 10 -15 minutes with stirring and let stand at this temperature ~~for~~ (60-65C) Filter through an asbestos lined gooch crucible. Wash filtrate with 1% HNO_3

Dissolve with ammonium hydroxide and hot water to a vol. of not more than 100 cc. Neutralize with HCl. Rotate slowly with 10 cc of Mg mixture. After 15 mins. add 12 cc ammonium. Stand 2 hrs. Filter and wash free of chlorides. Ignite to whiteness and weigh as Mg pyrophosphate.

PERIOD (1) EXCRETION OF CALCIUM AND PHOSPHORUS ON BASAL DIET ALONE.

INTERVAL	CALCIUM OXIDE			MAGNESIUM PYROPHOSPHATE		
	URINE	FECAL	TOTAL	URINE	FECAL	TOTAL.
2 days	.50	.55	1.05	5.77	1.38	7.15
2 days	.40	.80	1.20	4.1[illegible]	1.97	6.1[illegible]
2 days	.46	.38	.84	5.60	.91	6.50
2 days	135	.5[illegible]	.85	3.85	1.26	5.11
2 days	.39	.66	1.05	4.[illegible]	2.22	6.6[illegible]
2 days	.38	.57	.95	4.60	1.6[illegible]	6.20
2 days	.35	.65	1.00	5.81	2.37	8.10
14 days	2.83		6.94			45.67
per day	.50					3.2

PERIOD (2) EXCRETION OF CALCIUM AND PHOSPHORUS ON BASAL DIET PLUS BUTTER.

INTERVAL	CALCIUM OXIDE			MAGNESIUM PYROPHOSPHATE		
	URINE	FECAL	TOTAL	URINE	FECAL	TOTAL
2 days	.36	.33	.70	4.60	1.00	5.60
2 days	.78	.52	1.31	4.73	1.00	5.73
2 days	339	.27	.66	5.00	.39	[illegible].79
2 days	.48	.87	1.34	5.25	1.62	6.87
2 days	.41	.76	1.17	4.95	1.30	6.[illegible]5
2 days	.45	110	1.50	[illegible]	1.00	[illegible]
12 days	2.[illegible]8		6.68			34.[illegible]1
per day	.24		.56			2.9

PERIOD (3) EXCRETION OF CALCIUM AND PHOSPHORUS ON BASAL DIET [illegible].

	OXIDE OF CALCIUM			MAGNESIUM PYROPHOSPHATE		
interval	urinary	fecal	total	urinary	fecal	total
4 days	.76	2.51	3.27	12.5	2.8	15.3
4 days	.92	1.76	2.68	12.9	3.0	16.0
2 days	.45	1.80	2.25	4.16	3.04	7.2
10 days	2.13		8.20			38.5
per day	.21		.82			3.85

PERIOD (4) EXCRETION OF CALCIUM AND PHOSPHORUS ON BASAL DIET & COD LIVER OIL.

2 days	.378	2.32	2.70	5.4	3.0	8.4
2 days	.480	1.06	1.54	4.77	1.68	6.45
4 days	.64	1.61	2.25	9.00	2.80	11.80
4 days	.61	2.06	2.67	10.60	2.4	13.00
12 days	2.10		9.17			39.4
per day	.17		.76			3.3

Average daily weight of dried feces during:-

1st interval	2 nd period	3 rd period	4 th period
13 grams	11.2 grams	13.6 grams	12.5 grams.

Discussion.

The hypothesis of Doctor McCollum that cod liver oil exerts an osteoclastic action upon the shaft of the growing long bone arose from ~~the~~ his study upon the histology of the rachitic rat bones. Here he always noted that in rats killed after ~~a~~ living on a diet supplemented with cod liver oil, the shaft of the long bones showed a peculiar condition of porosity.

In planning this experiment to test the hypothesis that cod liver oil exerts an osteoclastic action we expected to find that the excretion of calcium would be about the same on both the basic diet and on the basic diet supplemented with butter fat. When, however, the basic diet was supplemented with cod liver oil a noticeable increase was expected. It was thought that this increase of calcium excretion would serve as chain evidence that calcium had come from the shaft of the long bones.

The results at hand show such small variations that one is hardly justified in drawing conclusions. Most assuredly we failed to obtain the expected increase.

The excretion of calcium on the butter fat diet showed a slight increase over the excretion of calcium on the simple basal diet. This increase was present in both the fecal and urinary output.

In the following periods the amount of food eaten was increased to prevent further loss of weight. This is brought out quite plainly by a comparison of the diets in the preceding pages. The amount of calcium excreted in the two succeeding periods is therefore greater than in the two preceding periods.

Comparing the period in which the basal diet alone was eaten with the period in which ~~the serie in which~~ cod liver oil was added to the basal diet it will be seen that the calcium excretion not only did not show a large increase with cod liver oil, but that it actually showed a slight decrease.

This unexpected finding is more or less in agreement with that of (21) B. Sjollema who after administering cod liver oil to rabbits found that there was a tendency on the part of the organism to hold back its calcium, no matter whether the food was rich or poor in calcium. There was a diminution in both renal and intestinal calcium excretion. With a diet rich in calcium the quanity of feces as well as their calcium content decreases if cod liver oil is given.

In carrying out our experiment no attemp was made to regulate the amount of exposure to sunlight. This in recent years has been found very important in the regulation of calcium metabolism in animals suffering with rickets. Recently Doctor Clarke has shown that radiation affects the amount or the combinations of dialyzable calcium in serum. This probably has a direct relation to the therapeutic effect of sunlight in calcium metabolism. If the experiment should be repeated it would seem advisable to control the amount of exposure to sunlight as well as other factors.

There is another point to be considered in this experiment. Nearly all the experiments so far have been carried out on young growing animals whose long bones were still in the process of growth. In this experiment the results were obtained from an adult in whom the length of the long bones had ceased to increase, i.e. the juncture of the head and shaft had calcified. It could easily be conceived that the effect of cod liver oil upon the deposition of calcium at the juncture of shaft and head would be quite different in the full grown bone than in the growing bone where there is constant need of calcium.

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